REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT DATE	3. DATES COVERED (From - To)
24/02/2004	Final Report	15/06/1998 to 14/06/2000

4. TITLE AND SUBTITLE
Production of Elastomeric Polypeptides for Materials Characterizations

5a. CONTRACT NUMBER
N00014-98-C-0279

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Urry, Dan W., Ph.D.

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Bioelastics Research, Ltd.
2800 Milan Court, Suite 386
Birmingham, AL 35211

8. PERFORMING ORGANIZATION REPORT NUMBER
BRL-0001Z

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy St.
Arlington, VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSORING/MONITORING AGENCY REPORT NUMBER

12. DISTRIBUTION AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
To prepare a family of designed elastic protein-based polymers for free energy transduction. Specifically, this effort is to provide 100 gram quantities of six and 10 gram quantities of an additional nine elastomeric polypeptides for conventional and specialized materials characterizations and for further development toward specific applications of potential use to the Navy.

15. SUBJECT TERMS
Elastic, protein-based polymers, inverse temperature transition, cross-link

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Timothy M. Parker
UU	UU	UU	UL	3	19b. TELEPONE NUMBER (Include area code) 205-943-6590

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI-Std Z39-18

FINAL REPORT

CONTRACT NUMBER: N00014-98-C-0279

CONTRACT TITLE: Production of Elastomeric Polypeptides for Materials Characterizations

PRINCIPAL INVESTIGATOR: Jie Xu, Ph.D. (e-mail:brl@oadi.uab.edu)

PI INSTITUION: Bioelastics Research, Ltd.

AWARD PERIOD: 15 June 1998 - 14 June 2000

LONG-TERM RESEARCH OBJECTIVE: The long term research objective is to develop the full capacity of the hydrophobic folding and assembly transition of elastic protein-based (amphiphilic) polymers in an aqueous milieu for catalyzing the diverse energy conversions of biology.

S&T OBJECTIVES: To prepare a family of designed elastic protein-based polymers for free energy transduction. Specifically, this effort is to provide 100 gram quantities of six and 10 gram quantities of an additional nine elastomeric polypeptides for conventional and specialized materials characterizations and for further development toward specific applications of potential use to the Navy.

APPROACH: By using genetic engineering approaches, the genes encoding various elastomeric polypeptides were constructed and engineered into *E coli* expression hosts. By having *E coli* expressing those gene products at adequate levels, it is possible to synthesize reproducible polymer materials in large quantities through a single fermentation run. The 500 or 28 liter fermentors are used to cultivate the cells and the materials are isolated from the cells employing their unique inverse temperature transitional (phase separation) properties.

ACCOMPLISHMENTS: Polymer production and cross-linking efforts.

The full set of polymers that were produced is listed below. They were produced at the desired quantities and transferred to University of Minnesota (UMN) for their studies. They were finished ahead of schedule.

Polymer	***I:**	$(GVGVP)_{251}$	
Polymer	***II:**	$(GVGIP)_{260}$	
Polymer	***III:**	$(GVGVP\ GVGFP\ G\underline{E}GFP\ GVGVP\ GVGFP\ GFGFP)_n(GVGVP)$	E/5F
Polymer	**°IV:**	$(GVGVP\ GVGFP\ G\underline{E}GFP\ GVGVP\ GVGFP\ GVGFP)_n(GVGVP)$	E/4F
Polymer	**°V:**	$(GVGVP\ GVGVP\ G\underline{E}GVP\ GVGVP\ GVGFP\ GFGFP)_n(GVGVP)$	E/3F
Polymer	**°VI:**	$(GVGVP\ GVGFP\ G\underline{E}GFP\ GVGVP\ GVGVP\ GVGVP)_n(GVGVP)$	E/2F
Polymer	***VII:**	$(GVGVP\ GVGVP\ G\underline{E}GVP\ GVGVP\ GVGVP\ GVGVP)_n(GVGVP)$	E/0F
Polymer	***VIII:**	$(GVGIP\ GFG\underline{E}P\ G\underline{E}GFP\ GVGVP\ GFGFP\ GFGIP\ GVGIP\ GFG\underline{E}P\ G\underline{E}GFP\ GVGVP\ GFGFP\ GFGIP)_n(GVGVP)$	2E/5F/2I
Polymer	**°IX:**	$(GVGVP\ GVGFP\ G\underline{K}GFP\ GVGVP\ GVGFP\ GFGFP)_n(GVGVP)$	K/5F
Polymer	**°X:**	$(GVGVP\ GVGFP\ G\underline{K}GFP\ GVGVP\ GVGFP\ GVGFP)_n(GVGVP)$	K/4F
Polymer	**°XI:**	$(GVGVP\ GVGVP\ G\underline{K}GVP\ GVGVP\ GVGFP\ GFGFP)_n(GVGVP)$	K/3F
Polymer	**°XII:**	$(GVGVP\ GVGFP\ G\underline{K}GFP\ GVGVP\ GVGVP\ GVGVP)_n(GVGVP)$	K/2F
Polymer	***XIII:**	$(GVGVP\ GVGVP\ G\underline{K}GVP\ GVGVP\ GVGVP\ GVGVP)_n(GVGVP)$	K/0F
Polymer	**°XIV:**	$(GVGVP\ GVGFP\ G\underline{E}GFP\ GVGVP\ GVGFP\ G\underline{K}GVP)_n(GVGVP)$	E/K/3F
Polymer	**°XV:**	$(GVGVP\ GVGFP\ G\underline{E}GFP\ GVGVP\ GVGVP\ G\underline{K}GVP)_n(GVGVP)$	E/K/2F

*Polymers supplied at 100 gram amounts. °Polymers supplied at 10 gram amounts. The polymers where completed by the end of September, which is months ahead of the revised and shortened timelines.

Large sheets of polymer I, II, III and VII were exposed to 20 Mrad of gamma-irradiation to form insoluble cross-linked matrixes and supplied to UMN.

A series of polymers, I, II, III, IV, VII, VIII and XIII, were cross-linked at varying doses of gamma irradiation. The compositions were exposed to 10, 14, 18, 22, 26, 30 and 34 Mrad for a total of seven cross-linked matrixes each. These were then supplied to UMN for their studies.

Also, additional fermentation runs were performed to produce materials to aid purification efforts by UMN and to demonstrate BRL levels of polymer expression. And a BRL scientist also visited UMN to assist fermentation efforts by UMN.

This set the stage for a more complete understanding of the physical basis for hydrophobic-induced shift in properties of functional groups.

CONCLUSIONS: All the polymers and materials proposed in the contract were made and delivered to the University of Minnesota. Additional and varied constructs where also prepared and supplied, once the need demonstrated. This was accomplished ahead of schedule so that UMN could complete their studies under the ONR contract number N00014-98-1-0656 which was running concurrently.

SIGNIFICANCE: There are numerous naval applications where free energy transduction is fundamental. One example would be in the development of MEMS (micro-electro-mechanical systems), which is directly related to stretch-induced free energy transductions.

TECHNOLOGY TRANSFER: The corporate entity, Bioelastics Research Ltd. (BRL), is already in place for the technology transfer. BRL holds basic patents to the polymer compositions and their use in a number of free energy transduction applications. An agreement between BRL and UMN governs the technology developed at UMN wherein BRL has exclusive rights for the technology derived during this effort.

REFERENCE:

D. W. Urry, S. Q. Peng, L. C. Hayes, D. McPherson, J. Xu, T. C. Woods, D. C. Gowda and A. Pattanaik, "Engineering Protein-based Machines to Emulate Key Steps of Metabolism (Biological Energy Conversion)" *Biotechnology and Bioengineering* **58(2&3)**, 175-190, 1998.

PUBLICATIONS ARISING FROM THE PRODUCTION OF POLYMERS FOR UMN

1. Jonghwi Lee, Frederic Prochazka, Dan W. Urry and Christopher W. Macosko, "Phase Transition and Elasticity of Protein-Based Hydrogels, J. Biomat. Sci. Polymer Edn., 12, 229-242, 2001.

2. Jonghwi Lee, Christopher W. Macosko, and Dan W. Urry, "Elastomeric Polypentapeptides Cross-Linked into Matrixes and Fibers," Biomacromolecules 2, 170-179, 2001.

3. Jonghwi Lee, Christopher W. Macosko, and Dan W. Urry, "Swelling Behavior of γ-Irradiation Cross-Linked Elastomeric Polypentapeptide-Based Hydrogels," Macromolecules, 34, 4114-4123, 2001.

4. Jonghwi Lee, Christopher W. Macosko, and Dan W. Urry, "Mechanical Properties of Cross-linked Synthetic Elastomeric Polypentapeptides," Macromolecules, 34, 5968-5974, 2001.

5. Jonghwi Lee, Christopher W. Macosko, and Dan W. Urry, "Elasticity of Polypentpeptide Hydrogels in Different Stress States", Polymeric Materials, Sci. & Eng., 84, 1005, 2001.